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The Gut and oral microbiome in HIV disease: Workshop B1

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Abstract

Recent years have seen a massive expansion in our understanding of how we interact with our microbial colonists. The development of new, rapid sequencing techniques such as pyrosequencing and other next-generation sequencing systems have enabled us to begin to characterize the constituents of our diverse microbial communities, revealing the astonishing genetic richness that is our microbiome. Despite this, our ignorance of how these communities change over the course of an HIV infection is profound. Whilst some steps have been made to characterize the HIV microbiome at selected sites, these reports are still limited and much remains to be done. It has become apparent, however, that host-microbiota interactions are perturbed during HIV infections, with microbial translocation of potential pathogens linked to a variety of different HIV complications, including more rapid progression of disease. The use of probiotics and prebiotics has been investigated as treatments to alleviate symptoms for a variety of conditions, and is now being proposed for the treatment of symptoms associated with HIV. However, this is a new area of investigations and many questions remain unanswered. What we know about both of these topics is a drop in the ocean compared with what we need to know. In this article, we report on a workshop where these two major under-investigated research areas were presented, and future directions explored and discussed.

Workshop Questions:

1.) What are the key characteristics of the oral microbiome in (HIV)-seropositive people? Are there differences in microflora related to HIV?

2.) Are probiotics promising agents in the prevention and/or care of HIV ?

Question 1:

What are the key characteristics of the oral microbiome in (HIV)-seropositive people? Are there differences in microflora related to HIV?

Studies into the host microbiome are an ever increasing field of study. A search of PubMed for the term 'microbiome' reveals that between 2000 and 2015, there were over 17,000 publications. The same search term indicated that in 2010, 1,171 publications were highlighted, whilst in 2014, this number increased to 4,855, indicating the massive expansion in interest in the field of microbiomics in recent years. However, despite this surge in interest, the majority of this work has explored either healthy individuals or chronic systemic conditions such as obesity, diabetes and inflammatory bowel diseases. In contrast, relatively few studies (183) have been carried out investigating the host microbiome during HIV infection, with the majority of these being more recent (64 publications in 2014). This is despite the well known and documented effects that HIV has on host immunity and associations with opportunistic infections. It is, therefore, somewhat surprising that so little effort has been made in investigating the changes in the microbiota after infection with HIV-1.

The Human Microbiome

It is now well established that mammalian cells make up a minority of the human body, with over 90% of the cells within or on our bodies being of microbial origin (Turnbaugh et al., 2007, Gill et al., 2006). The collective term coined for this wealth of microbial cells is the microbiota, whilst the sum of their genomes is referred to as the microbiome (Turnbaugh et al., 2007, Peterson et al., 2009). The bulk of the microbiota are usually regarded as commensal organisms, and exist in the various niches of the body with a degree of mutualism. However, in immunocompromised individuals, these otherwise non-pathogenic organisms may become opportunistic pathogens, and the term 'Pathobiont' has been coined to cover these organisms (Chow et al., 2011). The richness that the microbiome adds to the human genome is only now beginning to be realized. If, as has been proposed, rather than thinking of humans as a purely mammalian organism with microbial passengers, we think of ourselves as supraorganisms that encompass both human and microbial symbionts (Lederberg, 2000), then by far the majority of genes in this system are of microbial origin. Put in context, the 'mammalian' human has roughly the same number of genes as the *D.*

melanogaster fruit fly. However, when the human microbiome is added to the equation, this similarity is rapidly lost, as fruit flies, like many lower organisms, do not possess the complex microbiome possessed by humans (Gill et al., 2006, Turnbaugh et al., 2007). Thus, to gain a full understanding of the human genome, we must also characterize the human microbiome.

Recent attention to the human microbiota has been given to the observations of increased microbial translocation post HIV infection. These findings have identified an increase in the translocation of microbes and/or microbial products across epithelial surfaces, without an overt bacteraemia, occurring after damage to these surfaces. This suggests that deficiencies or defects in the immune component of mucosal surfaces, along with damage to the local environment, may affect systemic immunity during the chronic phase of an HIV infection, as surface microbes and microbial products translocate across these surfaces (Brenchley et al., 2006, Kanwar et al., 2010).Thus, given the interactions between the microbiota and the host along with its immune system, it is imperative that we gain a better understanding of how we interact with our microbiota in health and disease.

The human oral microbiome and HIV

A variety of oral manifestations have been associated with HIV infections and have been reviewed elsewhere (Medel & Hamao-Sakamoto, 2014). In general, the decrease in CD4 cell counts associated with HIV infection have been strongly linked with the presence of an increase in oral lesions (Hamza et al., 2006, Greenspan et al., 2004, Greenspan et al., 2001). The advent of successful treatment therapies for HIV - HAART (Highly Active Antiretroviral Therapy), appears to have had unexpected effects on oral infections and disease. Treatment of HIV seropositive patients with HAART has led to an increase in the incidence of human papilloma virus (HPV)-associated oral lesions and recurrent oral ulceration (Hamza et al., 2006, Ceballos-Salobrena et al., 2000), while the incidence of oral candidiasis and HIV-associated periodontal diseases have shown a decrease (Hamza et al., 2006, Greenspan et al., 2001, Greenspan et al., 2004). Although these changes have been linked to differences in access to oral health care, demographic and social factors and mode of HIV

transmission, other studies have also linked these changes to co-infection types, disease stages, immune reconstitution and composition of the oral microbiome - all aspects that will be affected by the local microbiota. It is therefore evident that the oral microbiota and its associated microbiome may change significantly as an HIV infection progresses.

A current clinical study has begun to characterize the microbiome in HIV infected individuals (Saxena et al., 2012, Li et al., 2014, Phelan et al., 2014). This study (Crosstalk among oral and gastrointestinal soluble innate factors, HIV and microbes: Grant U19 DE018385; www.nyu.edu/projects/crosstalk/) set out to examine a population of HIV-infected, antiretroviral-naïve subjects. The study has sampled the entire tract from mouth to anus, monitoring changes in proteome, microbiome and innate immune system, and preliminary results have yielded some interesting findings, relating to the microbiome during the course of an HIV infection event. Firstly, using 16S rDNA fingerprint profiling, the preliminary results indicate differences in profile between the healthy, uninfected group and the HIV-infected, antiretroviral-naïve group, with the infected group showing a greater microbial diversity as compared to the uninfected control group. This supports the hypothesis that HIV infection significantly changes the host microbiota, altering total microbial colonization and overall composition of the microbiota in the oral cavity. Using standard cultivation techniques to quantitatively and qualitatively evaluate the colonisation by several bacterial species, including *S. mutans*, *S. Sobrinus* and total *Lactobacillus* species, the preliminary results also indicate in increase in *Lactobacillus* and *S. mutans* in HIV-infected individuals as compared to the uninfected group. Also increased are the levels of the fungal *Candida* species, confirming that these normal constituents of a healthy microbiota show changes in their levels post-HIV infection. Confirmation has also been obtained using real-time PCR utilizing species-specific primers that there are changes in some of the primary pathogens associated with periodontitis. Analyzing the data using cluster analysis at the genus level indicated a unique microbiome in both groups, as HIV-infected and -uninfected individuals clustered separately. Taken together, these preliminary data indicate that on HIV infection there are significant shifts in the host microbiota that may explain some of the oral cavity

changes seen in HIV-infected individuals, such as increased *Candida* infections and increased periodontitis.

Given the changes in oral infection wrought by HAART treatment of HIV-infected individuals (e.g. decreases in the levels of periodontal disease and oral candidiasis), it is interesting to speculate what the effects of this treatment are on the oral microbiota changes observed in HIV infection. To this end, this study has now looked at HIV-infected patients before and six months following HAART therapy compared with healthy, uninfected individuals (Li et al., 2014). Using the same three methodologies (16s rDNA fingerprinting, cultivation and pyrosequencing), this study demonstrated differences in all three groups, indicating that HAART therapy also has an effect on the oral microbiota. As with the preliminary data, HIV-positive subjects had higher levels of total cultivable microbes, including oral S*treptococci*, *Lactobacilli*, *S. mutans* and *Candida* spp in saliva than did HIV-negative subjects. Further, these levels correlate with the CD4 T cell counts in these groups. A comparison of the 16S rDNA profiles indicated clear differences between the three different groups, with some genera (*Capnocytophaga, Slackia, Porphyromonas, Kingella, Peptostreptococcaceae, Lactobacillus,* and *Atopobium*) only detected in the HIV uninfected group. Interestingly, prevalence levels of the genera *Fusobacterium, Campylobacter, Prevotella, Capnocytophaga, Selenomonas, Actinomyces, Granulicatella*, and *Atopobium* increased again once an HIV infected patient had been receiving HAART. In contrast, prevalence of *Aggregatibacter* showed a significant decrease post HAART therapy. Taken together, these findings indicate that not only the infection by HIV, but also the subsequent treatment (HAART) can have a significant impact on the oral microbiota, affecting microbial colonization and composition. It is important to note that the rebound in microbiota seen for bacteria may not occur for the mycobiome (The fungal microbiome). A recent study comparing HIV-infected patients receiving HAART, compared with healthy controls indicated much the same findings as Li *et al* for the bacteriome, with few differences between the healthy and HAART-HIV groups (Mukherjee et al., 2014). Combined with the results of the study by Li *et al (Li et al., 2014)*, these data suggest that the oral bacteriome rebounds once HAART therapy starts. In contrast,

Mukherjee *et al* identified significant differences in the mycobiome between the healthy and HAART-HIV groups. In particular, they identified that *Pichia* prevalence was reduced, whilst prevalence of *Candida, Aspergillus* and *Cryptococcus* increased and, in doing so, identified a specific interaction between *Pichia* and these three pathogenic fungi. However, whist they have observed these effects, given that they have no data on the mycobiome in HIV-infected HAART-naive individuals, it is not clear if there is any reversion to the normal mycobiome in these patients, or if these individuals have a third, completely different mycobiome. In summary, whilst HIV infection clearly affects the oral microbiome, the effects on the oral bacteriome can be partially reversed by HAART therapy, although any reversal effects on the mycobiome remain to be defined.

Question 2:

Are probiotics promising agents in the prevention and/or care of HIV ?

As we have seen above, there is a growing body of evidence indicating the important role microbiota plays in our health. This has been manipulated over the years without our knowing it by the use of probiotics. A probiotic is a microbe that is believed to provide health benefits when ingested. Currently, the term is used to denote microbes that when consumed are associated with beneficial attributes to the host. The idea of probiotics is usually ascribed to Élie Metchnikoff who, in 1907, proposed that 'useful' microbes could be used to replace 'harmful' microbes in the gut and this process could be encouraged through the consumption of specific foods (Metchnikoff & Mitchell, 1907). Probiotics are now a global market, and claims for their benefits include decreasing the levels of harmful gut pathobionts, reducing gastro-intestinal discomfort, boosting the immune system, decreasing host pathogens, maintaining a 'healthy' microbiota during/post antibiotic treatment, along with a host of other, more esoteric benefits. The recent expansion in the sale and use of probiotics has resulted in an increase in the standards required to scientifically substantiate the

claimed beneficial effects of these agents (Rijkers et al., 2011), and to date, no conclusive studies have been published that reveal a cause-effect relationship, whilst their clinical efficacy remains to be conclusively proven.

As with microbiome research, this is a field in which there has been a recent surge in activity. A PubMed search using the search term 'probiotic' reveals that between 2000 and the end of 2014, 13,302 papers were published. However, the majority of this work has been done over the last five years. In 2000, only 212 papers were covered by the search term 'probiotic'. In contrast, in 2010, this number had surged to 1,239, and by 2014, the number was 1,961, indicating this is a healthy field that is ever expanding. Given the products that arise from this research and the companies that can retail them, such as food producers, there is also a significant amount of corporate research in this area that is driving investigations in several different fields of endeavor. However, investigations of the role of probiotics in HIV is an area of lower priority. A search of Pubmed using the term 'probiotic' and a disease name indicates that between 2000 and 2014, 1,224 papers were published investigating Inflammatory Bowel Disease and probiotics, 403 investigated Crohn's disease and probiotics and 565 were published investigating Irritable Bowel Syndrome and probiotics. In contrast, over the same time period, only 94 articles were published investigating probiotics and HIV, with over half of these published since 2010.

One of the main questions regarding the use of probiotics and HIV relates to the effects of probiotics on the host, given the increased prevalence of microbial translocation in HIV-infected individuals, and the effect of antiretroviral therapy (ART) on probiotics. The discovery that microbial translocation across mucosal surfaces can lead to increased inflammation and systemic immunity (Brenchley et al., 2006) indicated the importance that the microbiota can play in HIV infections. Systemic immunity activation, such as that induced by microbial translocation is associated with a range of non-infectious co-morbidities, all of which can result in a more rapid progression of the disease (Sandler et al., 2011, Ancuta et al., 2008). However, although systemic immune activation is

187 regarded as playing a role in maintaining the viral reservoir and speeding disease progression to AIDS
188 (Chomont et al., 2009, Hazenberg et al., 2003) there is contradictory evidence that despite adding to
189 a microbial load that can be translocated, probiotics may proffer a beneficial effect to HIV-infected
190 patients (Yang et al., 2014, Cunningham-Rundles et al., 2011) by redressing the balance of
191 commensals, pathobionts and pathogens resident at a mucosal surface, as well as inducing
192 improvements in epithelial barrier function. Thus, there is some debate about whether probiotics
193 are harmful or beneficial for HIV-infected individuals.

194 Probiotics have a largely ignored benefit in combating the microbiota changes that occur during
195 microbial translocation in the course of an HIV infection. However, several recent studies have
196 provided evidence to support a potential therapeutic role for these microbes - in particular VSL#3 (a
197 combination of strains of *Bifidobacteria, Lactobacilli* and *S. thermophila*) and *Lactobacillus*
198 *rhamnosus* that have been shown to have health benefits (Barclay, 1999, Corl et al., 2007), with
199 VSL#3 in particular showing improvements in Inflammatory bowel disease (IBD) (Bibiloni et al., 2005)
200 by improving barrier function of the GI tract (Sood et al., 2009). Likewise, *L. Rhamnosus* has been
201 shown to similarly improve IBD patient prognosis (Gupta et al., 2000). Given the ability of these
202 organisms to improve disease in IBD patients, it is therefore unsurprising and logical that *L.*
203 *Rhamnosus* has been found to have beneficial effects in HIV-infected individuals (Irvine et al., 2010).
204 More startling is the finding that this goes hand-in-hand with a marked increase in the levels of
205 peripheral $CD4^+$ T cells in conventional ART treated HIV-infected patients when given *L. Rhamnosus*
206 as a probiotic (Irvine et al., 2010). This effect is not limited to *L. Rhamnosus*, having recently been
207 seen with another probiotic (Yang et al., 2014).

208 Intimately linked with probiotics is the use of non-digestible food ingredients that can be used to
209 maintain specific microbiota constituents by promoting their growth and activity. These prebiotics,
210 usually oligosaccharides, directly alter the composition or indirectly affect the microbiota by
211 assorted effects on the host, such as modulation of gut immunity and changing intestinal transit rate

among others (Lomax & Calder, 2009, Lohner et al., 2014, Roberfroid et al., 2010). As such, it is possible that these agents could also impact on the health of an HIV-infected individual in the same ways as a probiotic. Indeed, in a pilot study of HAART-naïve HIV-infected patients, a specific prebiotic restored the gut microbiota, activated $CD4^+$ T cells and reduced microbial products (Gori et al., 2011). In summary, whilst little work has been done investigating the potential benefits of pro- and prebiotics for HIV-infected individuals, the small scale studies currently conducted suggest that there is potential in this area.

Future Directions

Whilst a good start has been made in investigating human microbiome during HIV infection, much work remains to be done. Given the great diversity seen between individual microbiota, greater numbers of individuals are needed to increase the sample size and thereby elucidate the core oral, gut respiratory, vaginal etc microbiota and microbiomes. Importantly, the interaction between the microbiota and host needs to be investigated. Currently, it is unclear what is causing the changes in microbiota associated with HIV infection. Furthermore, the status of the mycobiome has only recently begun to be investigated, and has revealed significant changes associated with HIV infection. An important issue that needs to be addressed in future studies is that of sample collection and patient characteristics. It is clear that the treatment status of HIV seropositive patients is critical in understanding the microbiota. Anti-retroviral therapies, in particular HAART, clearly have a significant impact on the microbiota. Likewise, length of infection, underlying co-infections, immune status and diet will all impact on the microbiota. Thus, sample collection needs to be standardized and full participant details are needed to make the most effective use of the data obtained. Attention to these issues would address the following questions:

Which comes first? The changes in the mucosa observed during HIV infection, or the changes in microbiota?

Are the changes in microbiota due to changes in the immune system, or are they independent of the immune state of the host?

What is the effect of the changes in microbiota on the oral, gut and vaginal mucosa?

What are the changes in the mycobiome and how do they relate to the progression of disease?

Despite promising early findings, the potential benefits of probiotics and prebiotics in treating either HIV infection or its symptoms is currently unclear. The potential that these agents hold for treating HIV-infected individuals is high. Given the potential availability and ease with which these agents can be mass-produced by low tech industries in the food production sector, they hold particular importance in less wealthy areas. However, an important note of caution should be sounded. Although these studies are promising, they are currently few, and the numbers enrolled in them are low. So, although the statistical analysis of them is promising, the benefits may not be as apparent in a larger sample size. Likewise, although a rebound in $CD4^+$ T cells was observed, this could be due to local inflammation, rather than a general rise in the number of these cells. Perhaps the biggest obstacle faced by these agents, however, is the lack of standardization. The clinical regulatory bodies of most nations (such as the FDA in the USA) regard these products as food products, meaning that there is a lack of regulation or licensing of them. As a result, most research in this area is carried out by food and nutrition laboratories and companies, rather than medical companies. Thus, although there is reason for investigation and interest, some important questions need to be answered.

Do probiotics change/alter the microbiota in HIV-infected individuals with and without standard treatments and therapies?

How do probiotics change/alter the immunology in HIV-infected individuals with and without standard treatments and therapies?

Do probiotics have a clinically beneficial effect in HIV-infected individuals with or without standard treatments and therapies?

Is there a reason not to give probiotics to HIV-infected individuals with or without standard treatments and therapies?

These major areas must be addressed to better understand both the make-up and the role of the microbiota in preventing/promoting HIV infection, along with treating both the disease and its associated symptoms. Through better understanding of the microbiota and how it can be manipulated, we will one day be able to use this knowledge to develop novel prophylactic strategies to alleviate many of the symptoms of HIV infection.

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